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Comparison of Chromatin Assays for DNA Fragmentation Evaluation in Human Sperm

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Sperm chromatin integrity is vital for successful pregnancy and transmission of genetic material to the offspring. We evaluated chromatin integrity in sperm from 60 infertile men and 7 fertile donors comparing the sperm chromatin structure assay (SCSA), TdT-mediated-dUTP nick end labeling (TUNEL), the sperm chromatin dispersion (SCD) test, and acridine orange staining technique (AOT). The TUNEL and SCD assays showed a strong relationship with the SCSA ($r > .866$; $P < .001$) for sperm DNA fragmentation, both in infertile men and donors of known fertility. AOT did not show any relationship with SCSA. The breakdown of the DNA fragmentation index (DFI) into 3 categories (≤15%, >15%-<30%, and ≥30%) showed that the SCSA, TUNEL, and SCD test predict the same levels of DNA fragmentation. AOT consistently showed higher levels of DNA fragmentation for each DFI category. DNA fragmentation in sperm between infertile men and donor sperm was significantly different ($P < .05$) under SCSA (22.0 ± 1.6 vs 11.8 ± 1.4), TUNEL (19.5 ± 1.3 vs 11.1 ± 0.9) and SCD (20.4 ± 1.3 vs 10.8 ± 1.1), respectively. DNA fragmentation in sperm evaluated by AOT did not differ ($P > .05$) between infertile men (31.3 ± 2.4) and donors (32.7 ± 4.8). AOT showed extreme variations for sperm DNA fragmentation in semen from both infertile men and donors. The problems of indistinct colors, rapid fading, and the heterogeneous staining were also faced. In conclusion, SCSA, TUNEL, and SCD show similar predictive values for DNA fragmentation, and AOT shows variable and increased levels of DNA fragmentation, which makes it of questionable value in clinical practice.

Key words: SCSA, TUNEL, SCD, AOT

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